

WHAT IS CLAIMED IS:

1. A nucleic acid comprising:

- 5 (a) a cleavage domain comprising a single-stranded region, said single-stranded region comprising at least one internucleotide linkage 3' to an adenosine residue, at least one internucleotide linkage 3' to a cytosine residue, at least one internucleotide linkage 3' to a guanosine residue, and at least one internucleotide linkage 3' to a uridine residue, and wherein said cleavage domain does not comprise a deoxyribonuclease-cleavable internucleotide linkage;
- 10 (b) a fluorescence reporter group on one side of the internucleotide linkages; and
- (c) a non-fluorescent fluorescence-quenching group on the other side of the internucleotide linkages.

15 2. The nucleic acid of claim 1, wherein the fluorescence-quenching group is a nitrogen- substituted xanthene compound, a substituted 4-phenyldiazenyl)phenylamine compound, or a substituted 4-(phenyldiazenyl)naphthylamine compound.

20 3. The nucleic acid of claim 1, wherein the fluorescence-quenching group is 4-(4'-dimethylaminophenylazo)benzoic acid), N,N'-dimethyl-N,N'-diphenyl-4-((5-t-butoxycarbonylaminopentyl) aminocarbonyl) piperidinylsulfonerhodamine, 4',5'-dinitrofluorescein, pipecolic acid amide, 4-[4-nitrophenyldiazinyl]phenylamine, or 4-[4-nitrophenyldiazinyl]naphthylamine.

25 4. The nucleic acid of claim 1, wherein the fluorescence reporter group is fluorescein, tetrachlorofluorescein, hexachlorofluorescein, rhodamine, tetramethylrhodamine, a Cy dye, Texas Red, a Bodipy dye, or an Alexa dye.

30 5. The nucleic acid of claim 1, wherein the fluorescence reporter group is attached to the 5'-terminal nucleotide of the nucleic acid.

6. The nucleic acid of claim 1, wherein the fluorescence quenching group is attached to the 5'-terminal nucleotide of the nucleic acid.

7. The nucleic acid of claim 1 which is a single-stranded RNA molecule.

8. The nucleic acid of claim 1 which is a chimeric oligonucleotide comprising a nuclease resistant modified ribonucleotide residue.

9. The nucleic acid of claim 8, wherein the modified ribonucleotide residue is an 2'-O-methyl ribonucleotide, a 2'-methoxyethoxy ribonucleotide, a 2'-O-allyl ribonucleotide, a 2'-O-pentyl ribonucleotide, or a 2'-O-butyl ribonucleotide.

10. The nucleic acid of claim 8, wherein the modified ribonucleotide residue is at the 5'-terminus or the 3'-terminus of the cleavage domain.

11. The nucleic acid of claim 1 which is a chimeric oligonucleotide comprising a deoxyribonuclease resistant modified deoxyribonucleotide residue.

12. The nucleic acid of claim 11, wherein the deoxyribonuclease resistant modified deoxyribonucleotide residue is a phosphotriester deoxyribonucleotide, a methylphosphonate deoxyribonucleotide, a phosphoramidate deoxyribonucleotide, a phosphorothioate deoxyribonucleotide, a phosphorodithioate deoxyribonucleotide, or a 5 boranophosphate deoxyribonucleotide.

13. The nucleic acid of claim 11, wherein the deoxyribonuclease resistant modified deoxyribonucleotide residue is in the cleavage domain.

14. The nucleic acid of claim 1 which comprises a ribonuclease-cleavable modified ribonucleotide residue.

15. The nucleic acid of claim 14, wherein the ribonuclease-cleavable modified ribonucleotide residue is in the cleavage domain.

16. The nucleic acid of claim 1 which is 5-30 nucleotides in length.
17. The nucleic acid of claim 16 which is 7-10 nucleotides in length.
- 5 18. The nucleic acid of claim 1, wherein the fluorescence-quenching group is 5' to the cleavage domain and the fluorescence reporter group is 3' to the cleavage domain.
19. The nucleic acid of claim 18, wherein the fluorescence-quenching group is at the 5' terminus of the nucleic acid.
- 10 20. The nucleic acid of claim 18, wherein the fluorescence reporter group is at the 3' terminus of the nucleic acid.
21. The nucleic acid of claim 1, wherein the fluorescence reporter group is 5' to the cleavage domain and the fluorescence-quenching group is 3' to the cleavage domain.
- 15 22. The nucleic acid of claim 21, wherein the fluorescence reporter group is at the 5' terminus of the nucleic acid.
- 20 23. The nucleic acid of claim 21, wherein the fluorescence-quenching group is at the 3' terminus of the nucleic acid.
24. The nucleic acid of claim 1, in which the cleavage domain comprises the formula: 5'-N₁-n-N₂-3', wherein:
- 25 (a) "N₁" represents zero to five 2'-modified ribonucleotide residues;
- (b) "N₂" represents one to five 2'-modified ribonucleotide residues; and
- (c) "n" represents four to ten unmodified ribonucleotide residues.
25. The nucleic acid of claim 24, wherein the fluorescence-quenching group "N₁" represents one to five 2'-modified ribonucleotide residues.
- 30 26. The nucleic acid of claim 25, wherein the fluorescence-quenching group is attached to the 5'-terminal 2'-modified ribonucleotide residue of N₁.

27. The nucleic acid of claim 25, wherein the fluorescence reporter group is attached to the 5'-terminal 2'-modified ribonucleotide residue of N₁.

5 28. The nucleic acid of claim 24, wherein the fluorescence-quenching group is 5' to the cleavage domain and the fluorescence reporter group is 3' to the cleavage domain.

29. The nucleic acid of claim 24, wherein the fluorescence reporter group is 5' to the cleavage domain and the fluorescence-quenching group is 3' to the cleavage domain.

10 30. The nucleic acid of claim 24, wherein the cleavage domain comprises the sequence "auggc".

31. The nucleic acid of claim 30, wherein N₁ and N₂ each represent one 2'-
15 modified ribonucleotide residue.

32. The nucleic acid of claim 31, wherein the 2'-modified ribonucleotide residue is an adenosine.

33. A kit comprising:

- 20 (a) in one container, a substrate, said substrate comprising a nucleic acid molecule comprising a single stranded region, said single-stranded region comprising
- i. a cleavage domain comprising a single-stranded region, said single-stranded region comprising at least one internucleotide linkage 3' to an adenosine residue, at least one internucleotide linkage 3' to a cytosine
25 residue, at least one internucleotide linkage 3' to a guanosine residue, and at least one internucleotide linkage 3' to a uridine residue, and wherein said cleavage domain does not comprise a deoxyribonuclease cleavable internucleotide linkage;
 - ii. a fluorescence reporter group on one side of the internucleotide
30 linkages; and
 - iii. a non-fluorescent fluorescence-quenching group on the other side of the internucleotide linkages.

34. The kit of claim 33, further comprising in a second container a ribonuclease.

35. The kit of claim 33, further comprising ribonuclease-free water.

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36. The kit of claim 33, further comprising a buffer.

37. The kit of claim 33, further comprising ribonuclease-free laboratory plasticware.

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38. A method for measuring the activity of a ribonuclease comprising the steps of obtaining a sample from which the activity of the ribonuclease is to be measured, mixing the sample with the nucleic acid of claim 1, and measuring the amount of fluorescence that is produced.

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39. The method of claim 38 wherein the step for measuring the amount of fluorescence produced is carried out by measuring fluorescence in a fluorimeter.

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